CARNA BIOSCIENCES

Carna Newsletter

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# DGKα and DGKζ are key targets for cancer immunotherapy

## DGKα and DGKζ are downregulators of TCR-mediated activity

One of the most recent innovations in cancer therapy is harnessing and awakening a patient's own immune system to target their tumor burden. These revolutionary methods include the expensive CAR-T cell therapies, but also include direct targeting of immune pathways with small molecules or biologics, which result in the enhancement of immune cell activity.

Two members of the diacylglycerol kinase (DGK) family have generated much recent interest for their role in downregulating T-cell responses, namely DGK α and DGKζ.

Diacylglycerol (DAG) is a potent intracellular secondary messenger produced by recruitment of phospholipase C gamma 1 (PLC-γ1) to the plasma membrane in response to engagement of a cell surface receptor. PLC-γ1 converts phosphatidylinositol 4,5-bisphosphate (PI45P2) to DAG and inositol trisphosphate (IP3). DGKs act to phosphorylate DAG to phosphatidic acid (PA), and therefore attenuate the DAG-mediated signal.

10 DGK isozymes are expressed in humans, with each having important physiological functions. Their aberrant functions are associated with a variety of pathologies[1–7] (Table.1). Among them, DGKα and DGKζ have been identified as critical regulators of T cell receptor (TCR) signaling.

Table.1. Pathologies associated with DGK isozymes

| | |
|---|---|
| **DGKα**: Cancer (Liver, Esophageal, Gastric, Pancreatic), Melanoma, Glioblastoma, Immune Diseases (X-linked lymphoproliferative disease 1, Localized juvenile periodontitis) | **DGKζ**: Osteosarcoma, Glioma, Immune Diseases (Asthma) |
| **DGKβ**: Bipolar Disorder | **DGKη**: Bipolar Disorder |
| **DGKγ**: Colorectal Cancer, Hepatocarcinoma | **DGKι**: Gastric Cancer |
| **DGKδ**: Type 2 Diabetes | **DGKθ**: Parkinson's Disease |
| **DGKε**: Epilepsy | **DGKκ**: Fragile X syndrome |

TCR activation by engagement with a compatible MHC/peptide complex, initiates rapid intracellular signaling cascades involving many proteins and resulting in a wide spectrum of cellular effects. One of these activated proteins is PLC-γ1, which as described above, generates DAG. DAG activates downstream signaling, including Protein Kinase C theta (PKCθ) and Ras guanyl nucleotide–releasing protein 1 (RasGRP1), resulting in activation of the Ras-ERK and PKC-NF-κB pathways, both of which are required for efficient transduction of the TCR signal[8][9] (Fig.1).

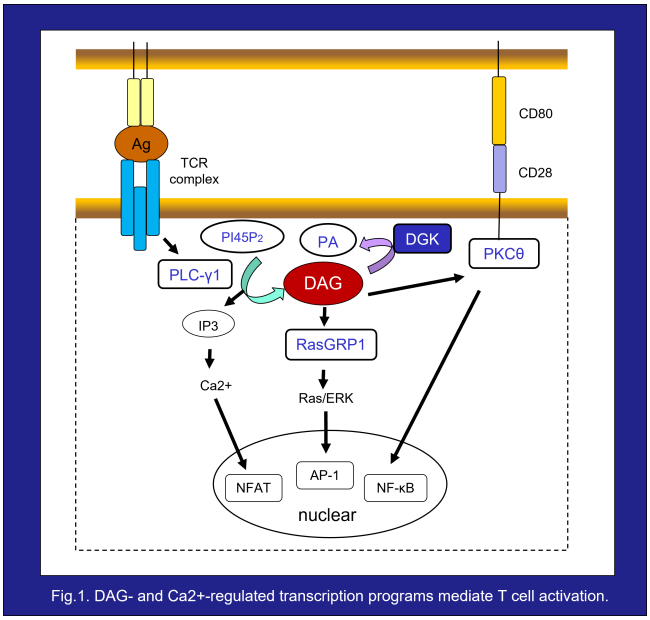


Fig.1. DAG- and Ca2+-regulated transcription programs mediate T cell activation.

## Targeting of DGKα and DGKζ in vitro and in vivo facilitates TCR-mediated signaling

Deletion of either DGKα or DGKζ isozymes results in enhanced ERK1/2 activation and increased functional responses of CD8+ T cells, including cellular proliferation and production of cytokines[10][11]. In cancer mouse models, DGKα-/- mice have been reported to show higher survival than WT mice, as well as an enhancement of survival with a co-treatment of an anti-PD1 biologic. In DGKζ-/- mice, the increased survival rate and the enhancement of survival with anti-PD1 treatment were shown not only in mice implanted with MC38 colon carcinoma

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cells (which are sensitive to monotherapy anti-PD1 treatment), but also B16F1 melanoma cells (which are minimally sensitive to treatment with anti-PD1)[12]. This suggests that DGKζ may be a good target for enhancing T cell anti-tumor activity in cancers resistant to anti-PD1 biologic therapy.

Sakane et al. reported the identification of CU-3 as a DGKα-selective inhibitor and that CU-3 induced T-cell activation[13], as well as DGKAI, an inhibitor of type I DGK isozymes (DGKα, DGKβ and DGKγ) which inhibits DGKα to a greater level than CU-3. DGKAI was also shown to suppress tumor growth in a hepatocellular carcinoma (HCC) mouse model, in a T-cell immune activity-dependent manner, and when combined with an anti-PD-L1 treatment, DGKAI showed synergistically enhanced anti-tumor activity[14].

These results suggest that inhibition of DGKα or DGKζ may enhance the T-cell anti-tumor activity and the efficacy of anti-PD1 or anti-PD-L1 treatments in a synergistic fashion by facilitating the activation of DAG-mediated TCR signaling. Therefore, the development of potent and specific inhibitors targeting these kinases is an attractive strategy for research on future oncology therapeutics.

## A full suite of DGK products & services from Carna

After a strategic pause following their initial release in 2016, all 10 DGK isozyme products (recombinant proteins) and services (screening and profiling) are now available from Carna to use in your research, from Sept 1st 2022 onwards.

Our background and knowledge of our DGK products and services is extensive with almost 10 years of experience with biotech and pharma. This is unique to the industry, and positions Carna as the most experienced partner for your DGK research (Fig.2).

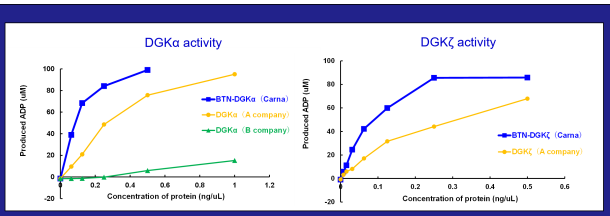


Fig.2. Comparison of DGK enzymatic activity for Carna's and competitor's products (ADP-Glo™ Kinase Assay, ATP=100μM)

Our kinases are manufactured in-house at Carna and delivered to you after stringent quality control. In addition, Carna offers assay kits and biochemical activity-based assay services using these active kinases.

Table 2. DGK product list

| Product Name | Catalog No. GST-tagged | Catalog No. Biotinylated (BTN-) |
|---|---|---|
| DGKα(DGKA) | 12-101 | 12-401-20N |
| DGKβ(DGKB) | 12-102 | 12-402-20N |
| DGKγ(DGKG) | 12-103 | 12-403-20N |
| DGKδ(DGKD) | 12-104 | 12-404-20N |
| DGKε(DGKE) | 12-115 | 12-415-20N |
| DGKζ(DGKZ) | 12-110 | 12-410-20N |
| DGKη(DGKH) | 12-106 | 12-406-20N |
| DGKθ(DGKQ) | 12-109 | 12-409-20N |
| DGKι(DGKI) | 12-107 | 12-407-20N |
| DGKκ(DGKK) | 12-108 | 12-408-20N |

If you are interested in Carna Biosciences' products & services, please contact us at info@carnabio.com.

- Lipid Kinase Proteins
- QuickScout Screening Assist™ ADP-Glo™ Assay Kits (Lipid Kinases)
- Activity-based Biochemical Profiling Assay Services (Lipid Kinases and others )

References：

1) Sci Signal. 2022; 15(729): eabo0264. Cooke M.
2) Clin Sci (Lond). 2020; 134(13): 1637-1658. Baldanzi G.
3) PLoS One. 2010; 5(10): e13447. Kakefuda K.
4) Cell. 2008; 132(3): 375-86. Chibalin AV.
5) Biochem Biophys Res Commun. 2005; 338(1): 77-81. Lukiw WJ.
6) Front Cell Dev Biol. 2016; 4: 82. Sakane F.
7) EMBO Mol Med. 2022; 14(5): e14649. Habbas K.
8) Sci Signal. 2015; 8(374): re6. Mérida I.
9) Mol Biol Cell. 2004; 15(6): 2932-42. Carrasco S.
10) J Biol Chem. 2011; 286(7): 5254-65. Riese MJ.
11) Nat Immunol. 2006; 7(11): 1174-81. Olenchock BA.
12) Oncoimmunology. 2021; 10(1): 1941566. Gu J.
13) J Lipid Res. 2016; 57(3): 368-79. Liu K.
14) Cancer Immunol Immunother. 2022; 71(4): 889-903. Okada N.

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